

TRADE SECRET

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STUDY TITLE: Primary Eye Irritation in Rabbits

TEST GUIDELINES: U.S. EPA Health Effects Test Guidelines, OPPTS 870.2400 (1998)
OECD Guidelines for Testing of Chemicals, Test No. 405 (2002)

**AUTHOR OF
ORIGINAL REPORT
AND REVISION NO. 1:**

DATES STUDY COMPLETED

ORIGINAL REPORT: November 18, 2010

REVISION NO. 1: November 24, 2010

**PERFORMING
LABORATORY:**

**LABORATORY
PROJECT ID:** Study Number

**WORK REQUEST
NUMBER:**

**SERVICE CODE
NUMBER:**

SPONSOR:

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are compatible with current OECD Good Laboratory Practices except for the item documented below. The item listed does not impact the validity of the study.

The test substance was characterized by the Sponsor prior to the initiation of this study. Although the characterization was not performed under Good Laboratory Practice Standards, the accuracy of the data is considered sufficient for the purposes of this study.

Sponsor:

Study Director:

11/24/10
Date

Sponsor: _____

Date

QUALITY ASSURANCE STATEMENT

The Quality Assurance Unit has reviewed this final study report to assure the report accurately describes the methods and standard operating procedures, and that the reported results accurately reflect the raw data of the study.

QA activities for this study:

QA Activity	Date Conducted	Date Findings Reported To Study Director And Management
Protocol review	Apr 23, 2008 ¹ ; Oct 27, 2010	Apr 23, 2008; Oct 27, 2010
In-process inspection: <i>72-hour scoring for animal #3401 and Day 2 in-life observations for animals #3402 and 3403</i>	Sept 16, 2010 and Sept 23, 2010	Oct 27, 2010
Raw data audit	Oct 27, 2010	Oct 27, 2010
Draft report review	Oct 27, 2010	Oct 27, 2010
Original final report review	Nov 18, 2010	Not applicable

11/24/10
Date

¹ The protocol used for this study was reviewed by the Quality Assurance group on this date.

CERTIFICATION

I, the undersigned, declare that the methods, results and data contained in this report faithfully reflect the procedures used and raw data collected during the study.

11/24/2010

Date

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STUDY INFORMATION

Substance Tested:

Number:

Composition:

Purity: See composition, above

Physical Characteristics:

Study Initiated/Completed: August 16, 2010 / (see report cover page)

Experimental Start/Termination: September 13, 2010 / September 24, 2010

In-Life Initiated/Completed: September 13, 2010 / September 24, 2010

Notebook Number(s):

PRIMARY EYE IRRITATION IN RABBITS

PROTOCOL NO.:

AGENCY:

EPA (TSCA) and OECD

STUDY NUMBER:

SPONSOR:

SPONSOR REPRESENTATIVE:

SPONSOR STUDY MONITOR:

TEST SUBSTANCE IDENTIFICATION:

DATE RECEIVED:

August 12, 2010

REFERENCE NO.:

STUDY INITIATION DATE:

August 16, 2010

EXPERIMENTAL INITIATION DATE:

September 13, 2010

EXPERIMENTAL COMPLETION DATE:

September 24, 2010

STUDY COMPLETION DATE:

November 18, 2010

1. PURPOSE

To provide information on the potential eye irritation from a single instillation to

2. SUMMARY

A primary eye irritation test was conducted with rabbits to determine the potential for to produce irritation from a single instillation via the ocular route.

At the request of the Sponsor, the study was conducted in a stepwise fashion. Initially, one-tenth of a milliliter of the test substance was instilled into the conjunctival sac of the right eye of one healthy rabbit by pulling the lower lid away from the eyeball. The upper and lower lids were then

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gently held together for about one second before releasing to minimize loss of the test substance. The left eye remained untreated and served as a control. Ocular irritation was evaluated by the method of Draize *et al.*¹ (see Table 4). Since irritation cleared by 24 hours and there was no significant irritation observed in this animal, the test was completed on two additional animals, as described above.

There was no corneal opacity or iritis observed in any treated eye during the study. Conjunctival redness (score of 1) and discharge (scores of 1 or 2) were observed in all treated eyes. All animals were free of ocular irritation by 48 hours.

In accordance with the provisions of Directive 1999/45/EC, classification is not required based on the results of this study.

According to the Globally Harmonized System (GHS) of classification and labeling of chemicals and under the conditions of this study, classification is not required.

3. MATERIALS

A. Test Substance

The test substance, identified as _____, was received on August 12, 2010 and was further identified with _____ Reference Number _____. The test substance was stored at room temperature. The sample was instilled as received. Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by the Sponsor.

The following information related to the test substance was provided by the Sponsor:

Physical description:

pH:

Solubility: Not provided

Stability: The test substance was expected to be stable for the duration of testing

Expiration Date: March 1, 2011

The test substance will be retained for at least 3 months following submission of the final report, unless otherwise specified by the Sponsor. After this time period all remaining test substance will be properly disposed. Records of sample disposition are maintained by _____

B. Animals

3.B.1 Number of Animals: 3

3.B.2 Sex: Female, nulliparous and non-pregnant

3.B.3 Species/Strain: Rabbit/New Zealand albino.

3.B.4 Age: Young adult.

¹ Draize, J.H., Woodward, G. and Calvery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Ther.* 1944; 82:377-390.

- 3.B.5 Source: Received from Robinson Services, Inc. Clemmons, NC on September 8, 2010.

4. METHODS

A. Husbandry

- 4.A.1 Housing: The animals were singly housed in suspended stainless steel caging with mesh floors which conform to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals* (Natl. Res. Council, 1996). Enrichment (e.g. toy) was placed in each cage. Litter paper was placed beneath the cage and was changed at least three times per week.
- 4.A.2 Animal Room Temperature and Relative Humidity Ranges: 19-22 °C and 43-69%, respectively.
- 4.A.3 Photoperiod: 12 hour light/dark cycle
- 4.A.4 Acclimation Period: 5 or 13 days
- 4.A.5 Food: Pelleted Purina Rabbit Chow #5326
- 4.A.6 Water: Filtered tap water was supplied *ad libitum* by an automatic water dispensing system.
- 4.A.7 Contaminants: There were no known contaminants reasonably expected to be found in the food or water at levels which would have interfered with the results of this study. Analyses of the food and water are conducted regularly and the records are kept on file at

B. Identification

- 4.B.1 Cage: Each cage was identified with a cage card indicating at least the study number and identification and sex of the animal.
- 4.B.2 Animal: A number was allocated to each rabbit on receipt and a stainless steel ear tag bearing this number was attached to the animal. This number, together with a sequential animal number assigned to study constituted unique identification.

5. PROCEDURE

A. Preparation and Selection of Animals

Prior to test initiation, both eyes of a group of animals were examined using a white light source and a fluorescein dye procedure. One drop of 2% ophthalmic fluorescein sodium was instilled into both eyes of each rabbit. The eyes were rinsed with physiological saline (0.9% NaCl) approximately 30 seconds after instillation of the fluorescein and then evaluated for corneal damage using an ultraviolet light source. Within 24 hours prior to instillation, the eyes were re-examined and scored for abnormalities according to the "Scale for Scoring Ocular Lesions" (see Table 4). Three healthy naive animals (not previously tested) without pre-existing ocular irritation was selected for test.

Initially, only one rabbit was placed on test. In the absence of significant irritation in this animal, the remaining two animals were tested to confirm the result.

B. Instillation

Prior to instillation, two to three drops of ocular anesthetic (Tetracaine Hydrochloride Ophthalmic Solution, 0.5%) were placed into both the treated and control eye of each animal. One-tenth of a milliliter of the test substance was then instilled into the conjunctival sac of the right eye of each rabbit by pulling the lower lid away from the eyeball. The upper and lower lids were then gently held together for about one second before releasing to minimize loss of the test substance. The other eye of each rabbit remained untreated with the test substance and served as a control. The rabbits were then returned to their designated cage.

C. Ocular Scoring

Ocular irritation was evaluated using a high-intensity white light (Mag Lite) in accordance with Draize *et al.*¹ (see Table 4) at 1, 24, 48, and 72 hours post-instillation. The fluorescein dye evaluation procedure described in Section 5.A. was used in the treated eye at 24 hours to verify the absence of corneal damage. Individual scores were recorded for each animal. In addition to observations of the cornea, iris and conjunctivae, any other observed lesions were noted.

D. Classification of Eye Scores

The observed ocular effects were classified as follows:

European Economic Community (EEC)

(Classification based on the mean scores of the cornea, iris, or conjunctiva averaged across 24, 48 and 72 hrs for all animals)

R-36 Risk Phrase and “Xi” Symbol..... Irritating to Eyes

Assigned if, when applied to the eye of the test animal, the test substance causes significant ocular lesions which occur within 72 hours after exposure and which persist for at least 24 hours. Ocular lesions are significant if the individual animal mean values of the 24-, 48-, and 72-hour evaluations in 2 or more animals comply with any of the following criteria:

- | | |
|-------------------------|---|
| - corneal opacity | equal to or greater than 2.0, but less than 3.0 |
| - iridial lesion | equal to or greater than 1.0, but less than 2.0 |
| - conjunctival redness | equal to or greater than 2.5 |
| - conjunctival chemosis | equal to or greater than 2.0 |

R-41 Risk Phrase and “Xi” Symbol..... Severe Damage to the Eyes

Assigned if the test substance causes severe ocular lesions which occur within 72 hours after exposure and which persist for at least 24 hours. Ocular lesions are severe if the individual animal mean value of the 24-, 48-, and 72-hour evaluations in 2 or more animals comply with any of the following criteria:

¹ Draize, J.H., Woodward, G. and Calvery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Ther.* 1944; 82:377-390.

Primary Eye Irritation in Rabbits

- | | |
|-------------------|------------------------------|
| - corneal opacity | equal to or greater than 3.0 |
| - iridial lesion | equal to 2.0 |

also required if:

- ocular lesions are still present 21 days following treatment
- the test substance causes irreversible coloration of the eyes

(Reference: European Communities Directive on Classification, Packaging and Labeling of Dangerous Substances (June 27, 1967), 67/548/EEC and subsequent amendments)

Globally Harmonized System (GHS)

Mean values for each lesion (corneal opacity, iris, conjunctival redness, conjunctival chemosis) were calculated for each animal separately from numerical scores obtained at the 24-, 48-, and 72-hour observations. The results were interpreted according to United Nations Economic Commission for Europe, Globally Harmonized System of Classification and Labeling of Chemicals (GHS), Part 3 - Health Hazards.

Category 1 (irreversible effects on the eye) is a test substance that produces:

- at least in one animal effects on the cornea, iris, or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days, and/or
- at least in 2 of 3 tested animals, a positive response of:
corneal opacity ≥ 3 and/or
iritis > 1.5
- calculated as the mean scores following grading at 24, 48, and 72 hours after instillation of the test substance

Category 2 (reversible effects on the eye) (irritating to eyes)

Category 2A (irritating to eyes) is a test substance that produces:

- at least in 2 of 3 tested animals a positive response of:
corneal opacity ≥ 1 , and/or
iritis ≥ 1 , and/or
conjunctival redness ≥ 2 , and/or
conjunctival chemosis ≥ 2
- calculated as the mean scores following grading at 24, 48, and 72 hours after instillation of the test substance, and
- which fully reverses within an observation period of normally 21 days

Within this category an eye irritant is considered **mildly irritating to eyes (Category 2B)** when the effects listed above are fully reversible within 7 days of observation.

E. Body Weights

Individual body weights of the animals were recorded prior to test substance application (initial) and again on the last day of scoring.

F. Clinical Observations

The animals were observed for signs of gross toxicity and behavioral changes at least once daily during the test period.

6. ANIMAL WELFARE ACT COMPLIANCE

This study complied with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR) and the Guidelines from the Guide for the Care and Use of Laboratory Animals (Nat'l. Res. Council, 1996). All studies conducted for adhere to the following principles:

- The sponsor ensures that the study described in this report does not unnecessarily duplicate previous experiments, and is in compliance with the Policy on Animal Testing.
- Whenever possible, procedures used in this study have been designed to implement a reduction, replacement, and/or refinement in the use of animals in an effort to avoid or minimize discomfort, distress or pain to animals. All methods are described in this study report or in written laboratory standard operating procedures.
- policy is that animals experiencing severe pain or distress that cannot be relieved are painlessly euthanized, as deemed appropriate by the veterinary staff and study director or appropriate designee.
- Methods of euthanasia used during this study were in conformance with the above referenced regulation and the recommendations of the American Veterinary Medical Association (AVMA), 2007 Guidelines on Euthanasia.
- Animals were provided with species-appropriate environmental enrichment.

is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

7. STUDY CONDUCT

This study was conducted at

The study director for this study was
The primary scientist for this study was
contributions by .

8. TEST GUIDELINES

The procedures as described in the protocol are based on the following testing guidelines:

U.S. EPA Health Effects Test Guidelines, OPPTS 870.2400 (1998)

OECD Guidelines for Testing of Chemicals, Test No. 405 (2002)

9. QUALITY ASSURANCE

The final report was audited for agreement with the raw data records and for compliance with the protocol and Standard Operating Procedures. Dates of inspections and audits

performed during the study, and the dates of reporting of the inspection and audit findings to the Study Director and Facility Management are presented in the Quality Assurance Statement.

10. DEVIATIONS FROM THE FINAL PROTOCOL

None.

11. FINAL REPORT AND RECORDS RETENTION

A copy of the signed report, copies of all raw data generated at _____ and the original signed protocol and amendments (if any), will be maintained in the _____ archives. _____ will maintain these records for a period of at least five years.

Laboratory-specific or site-specific raw data, such as personnel files and equipment records will be retained by the facility where the work was done.

Specimens (if applicable), raw data, and a copy of the protocol and amendments (if any), and the final report will be retained at _____

12. RESULTS

Individual body weights are presented in Table 1. Individual eye irritation scores are presented in Table 2. A summary of mean scores is presented in Table 3. The Draize Primary Eye Irritation Scoring System is presented in Table 4.

All animals appeared active and healthy and gained body weight during the study. Apart from the eye irritation noted below, there were no other clinical signs observed.

There was no corneal opacity or iritis observed in any treated eye during the study. Conjunctival redness (score of 1) and discharge (scores of 1 or 2) were observed in all treated eyes. All animals were free of ocular irritation by 48 hours.

13. CONCLUSION

In accordance with the provisions of Directive 1999/45/EC, classification is not required based on the results of this study.

According to the Globally Harmonized System (GHS) of classification and labeling of chemicals and under the conditions of this study, classification is not required.

TABLE 1: INDIVIDUAL BODY WEIGHTS

Animal No.	Sex	Body Weight (g)	
		Initial	Terminal
3401	F	2368	2379
3402	F	2189	2278
3403	F	2253	2352

TABLE 2: INDIVIDUAL SCORES FOR OCULAR IRRITATION

	Rabbit No.: 3401 (Female)				Rabbit No.: 3402 (Female)				Rabbit No.: 3403 (Female)			
	Hours				Hours				Hours			
	1	24	48	72	1	24	48	72	1	24	48	72
I. Cornea												
A. Opacity	0	0 ¹	0	0	0	0 ¹	0	0	0	0 ¹	0	0
B. Area	4	4	4	4	4	4	4	4	4	4	4	4
II. Iris												
A. Values	0	0	0	0	0	0	0	0	0	0	0	0
III. Conjunctivae												
A. Redness	1	0	0	0	1	0	0	0	1	1	0	0
B. Chemosis	0	0	0	0	0	0	0	0	0	0	0	0
C. Discharge	1	0	0	0	2	0	0	0	1	0	0	0

¹ 2% ophthalmic fluorescein sodium used to verify the absence of corneal opacity.

TABLE 3: MEAN SCORES FOR INDIVIDUAL RABBITS

Rabbit Number	Corneal Opacity^a	Iritis^a	Conjunctival Redness^a	Conjunctival Chemosis^a
3401	0.0	0.0	0.0	0.0
3402	0.0	0.0	0.0	0.0
3403	0.0	0.0	0.3	0.0
a Calculated from the 24-, 48-, and 72-hour scores (EEC/GHS).				

TABLE 4: SCALE FOR SCORING OCULAR LESIONS¹

1. Cornea	
A. Opacity-degree of density (area most dense taken for reading)	
No Opacity.....	0
Scattered or diffuse area, details of iris clearly visible	1 ²
Easily discernible translucent areas, details of iris slightly obscured	2 ²
Opalescent areas, no details of iris visible, size of pupil barely discernible	3 ²
Opaque, iris invisible.....	4 ²
B. Area of cornea involved	
One quarter (or less) but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters.....	3
Greater than three quarters, up to whole area	4
2. Iris	
A. Values	
Normal.....	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1 ²
No reaction to light, hemorrhage, gross destruction (any or all of these).....	2 ²
3. Conjunctivae	
A. Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)	
Vessels normal.....	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible.....	2 ²
Diffuse beefy red	3 ²
B. Chemosis	
No swelling.....	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2 ²
Swelling with lids about half-closed.....	3 ²
Swelling with lids about half-closed to completely closed.....	4 ²
C. Discharge	
No discharge.....	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals).....	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

¹ Draize, J.H., Woodward, G. and Calvery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharmacol. Exp. Ther. 1944; 82:377-390.

² These scores represent a positive response.

APPENDIX A: REVISION 1 EXPLANATION

The report was revised as follows:

<u>Page(s)</u>	
6	The test substance tested and 1st ingredient under composition on the Study Information page was changed.
18	Appendix A: Revision 1 added.

The following pages were revised to reflect these changes.

<u>Page</u>	
1	Title Page
5	Table of Contents